Docket No.: 050229-0415 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Customer Number: 20277
Jayakrishna AMBATI	:	Confirmation Number: 6795
Application No.: 10/685,705	:	Group Art Unit: 1632
Filed: October 16, 2003	:	Examiner: Anne Marie FALK

For: METHODS AND ANIMAL MODEL FOR ANALYZING AGE-RELATED MACULAR DEGENERATION

DECLARATION OF JAYAKRISHNA AMBATI UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, JAYAKRISHNA AMBATI, declare as follows:

1. I am the named inventor on the above-captioned patent application directed to animal models for analyzing age-related macular degeneration (AMD).

2. I am familiar with the prosecution of the above-captioned and the statements made by the Examiner in Office Action mailed October 12, 2006 and the with respect to the claims at issue being rejected under 35 U.S.C. § 112 first paragraph for allegedly failing to reasonably provide enablement for methods of testing candidate drugs for the treatment or prevention of AMD, using single(Ccl2-/- or Ccr2-/-) or dual knockout mice.

3. Dual knockout mice have been created in my laboratory by crossing the single knockout mice, Ccl2-/- and Ccr2-/- as described in the above-referenced patent application on page 20. Briefly, a Ccr2-/- mouse was mated with a Ccl2-/- mouse to yield heterozygous

progeny. The F1 mice were intercrossed and the progeny screened by PCR for the presence of the Ccl2 and Ccr2 genotype. B1 progeny, heterozygous for Ccr2 and Ccl2 were intercrossed, and mice homozygous for both genes were identified by PCR typing for continued backcrossing. The progeny mice were genotyped and their eyes were analyzed for evidence of the development of drusen.

4. Figures 1-6, enclosed herewith, demonstrate the phenotype of the dual knockout mice. Figure 1, is a photograph of agarose gel electrophoresis from PCR-based genotype assays of Ccr2, MCP1 and Ccr2/MCP1 knockout mouse strains. **Top.** In Ccr2 knockout gel, lane 1 is a 100bp DNA ladder; DNA from wild-type C57BL6/ mice (WT) is shown in lanes 2 and 8 with amplicon band at 400bp marker; DNA from 5 different Ccr2-/- mice (ko) is in lanes 3-7 with expected amplicon band at 450bp; and DNA from a heterozygous Ccr2+/- mouse (Het) is represented in lane 9 with two amplicon bands at 400bp and 450bp. **Middle.** In the MCP-1 knockout gel, lane 1 is a 100bp ladder;lane 3 is WT DNA with appropriate amplicon band at 895bp; lanes 2, 7, and 8 demonstrate MCP1-/- mice(ko) with expected amplicon of 430bp; and lanes 3,5 and 6 show two amplicon bands at 430bp and 895bp consistent with mice heterozygous for MCP1. **Bottom.** In the Ccr2/MCP1 double knockout gel, DNA from 4 different animals (C786, C787, C857, and C858) in our breeding scheme was used to test for MCP1 and Ccr2 mutants. On the left, all DNA samples show mutant amplicon at 450bp confirming that these mice are Ccr2-/-. DNA samples from the same animals were also assayed for MCP-1 mutants, as shown on the right side of this gel, with the expected amplicon at 430bp confirming that these mice are double knockouts for the Ccr2 and MCP-1 genes.

Figure 2 is a photograph of 4-week-old CCL2 x CCR2 double knockout mouse's retina showing multiple yellow-white subretinal deposits, i.e., drusen.

Figure 3 is a photograph of 6-week-old CCL2 x CCR2 double knockout mouse's retina showing multiple yellow-white subretinal deposits (drusen) and small triangular patches corresponding to geographic atrophy of the retina.

Figure 4 is a photograph of 12-week-old CCL2 x CCR2 double knockout mouse's retina showing multiple patchy areas of widespread geographic atrophy of the retina.

Figure 5 is an electronic micrograph of 8-week-old CCL2 x CCR2 double knockout mouse's retina showing deposition of debris between the retinal pigmented epithelium and Bruch's membrane (arrowhead) indicating the presence of confluent, "soft" drusen.

Figure 6 is an electronic micrograph of 8-week-old CCL2 x CCR2 double knockout mouse's retina showing deposition of debris between the retinal pigmented epithelium and Bruch's membrane (bracket) indicating the presence of confluent, "soft" drusen as well as focal nodular excrescence (arrowheads) indicating the presence of "hard" drusen.

The figures clearly demonstrate that dual knockout mice have been generated and that such mice have the expected phenotype.

5. I hereby declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

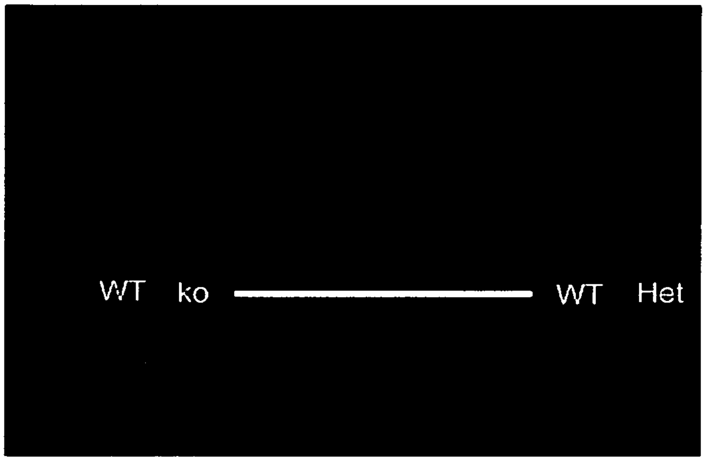
Signed this 11 day of April, 2007

A. Jayakrishna

Jayakrishna Ambati, M.D.

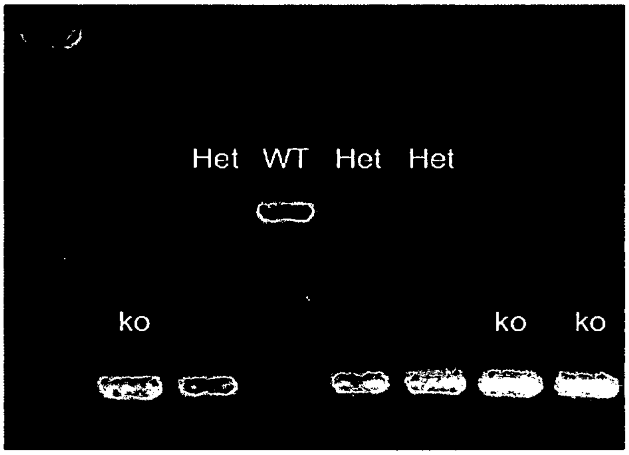
Figure 1

Ccr2 knockout



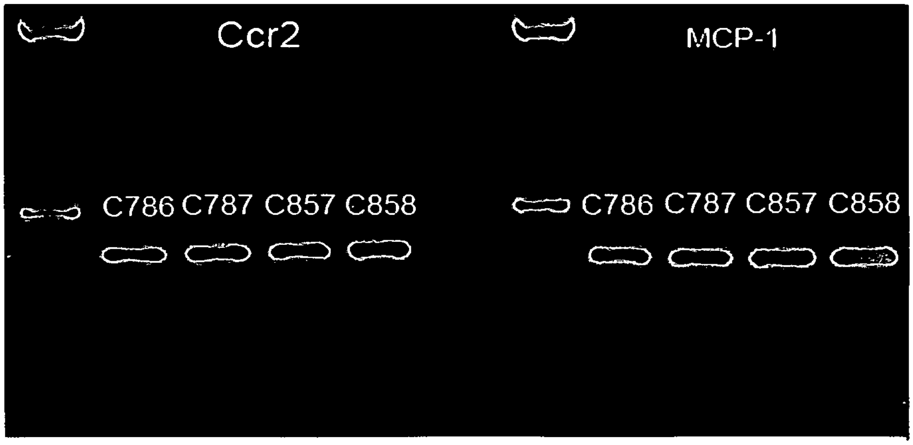
WT Amplicon 400bp
Ccr2 knockout Amplicon 450bp

MCP-1 knockout



WT Amplicon 895bp
Knockout Amplicon 430bp

Ccr2/MCP-1 double knockout



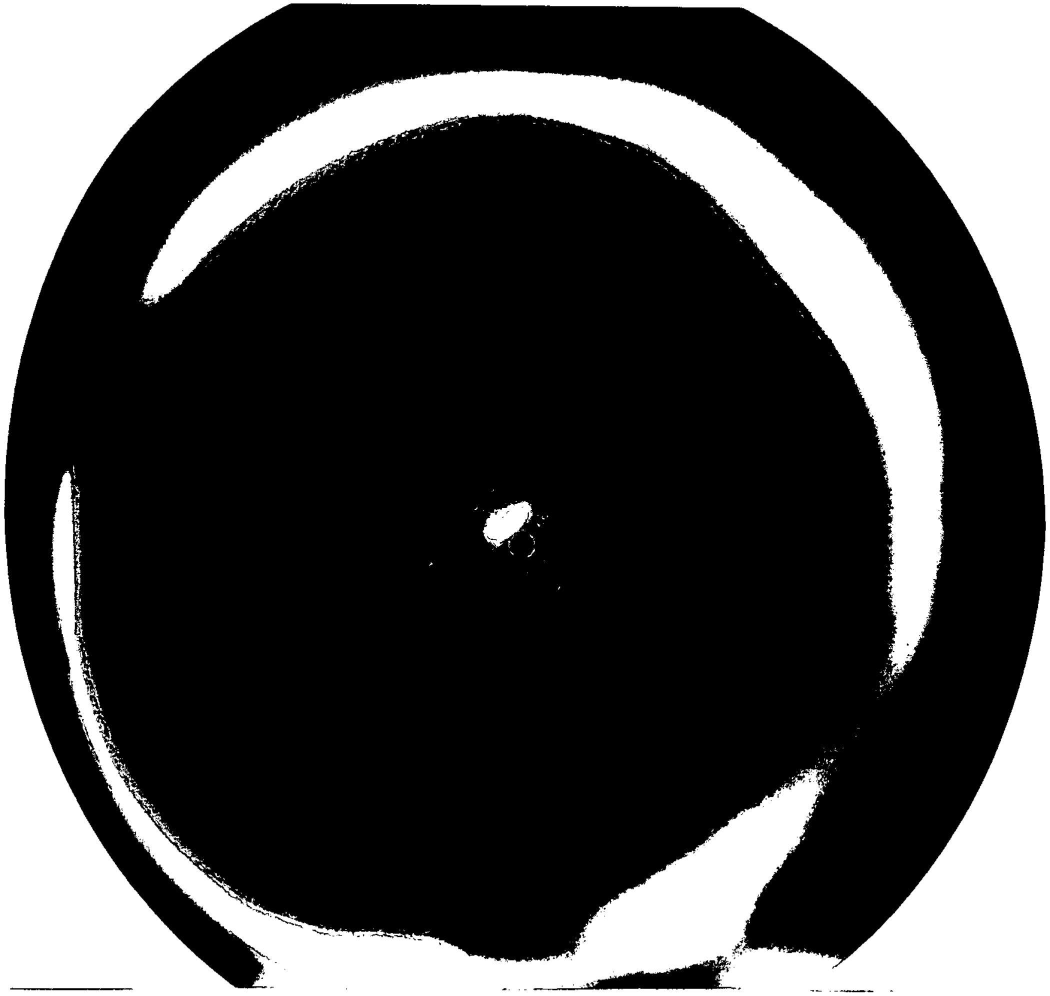


Figure 2

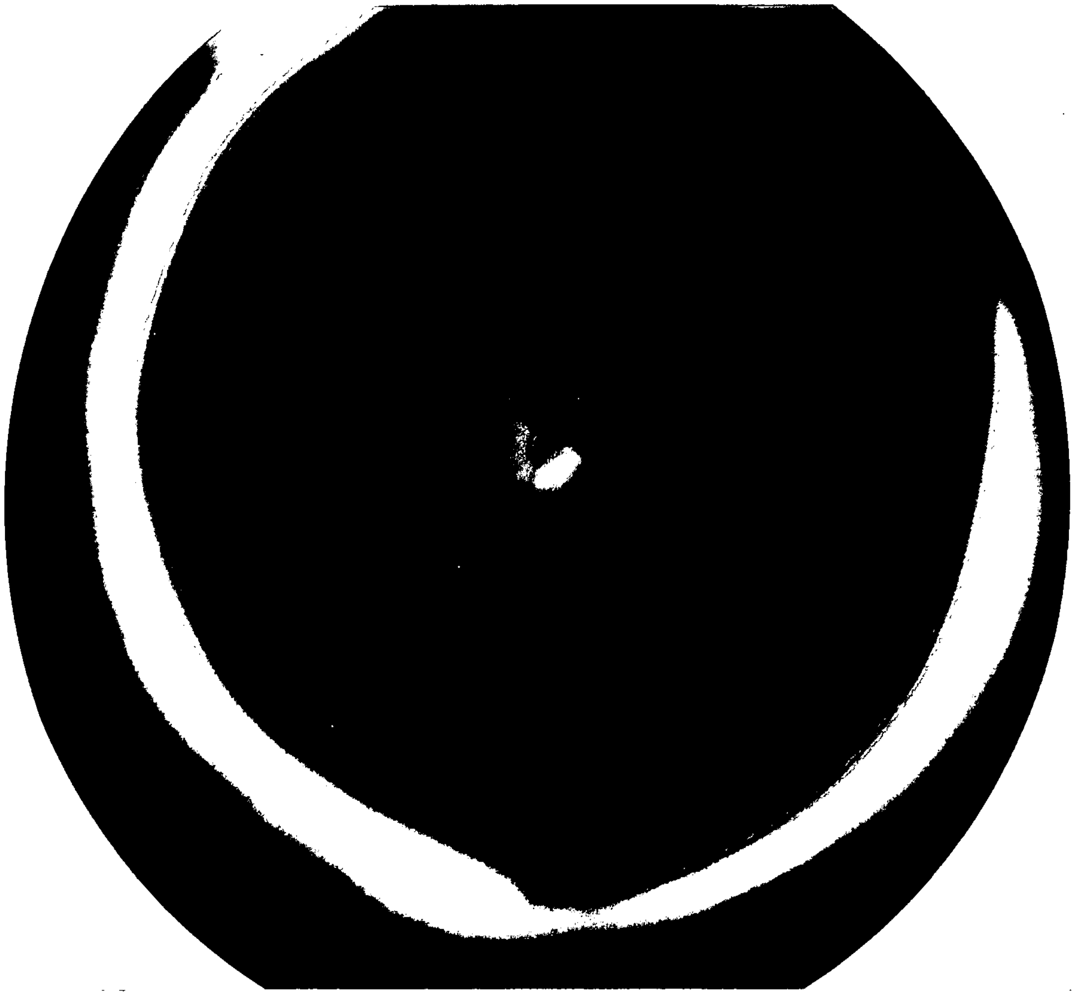


Figure 3

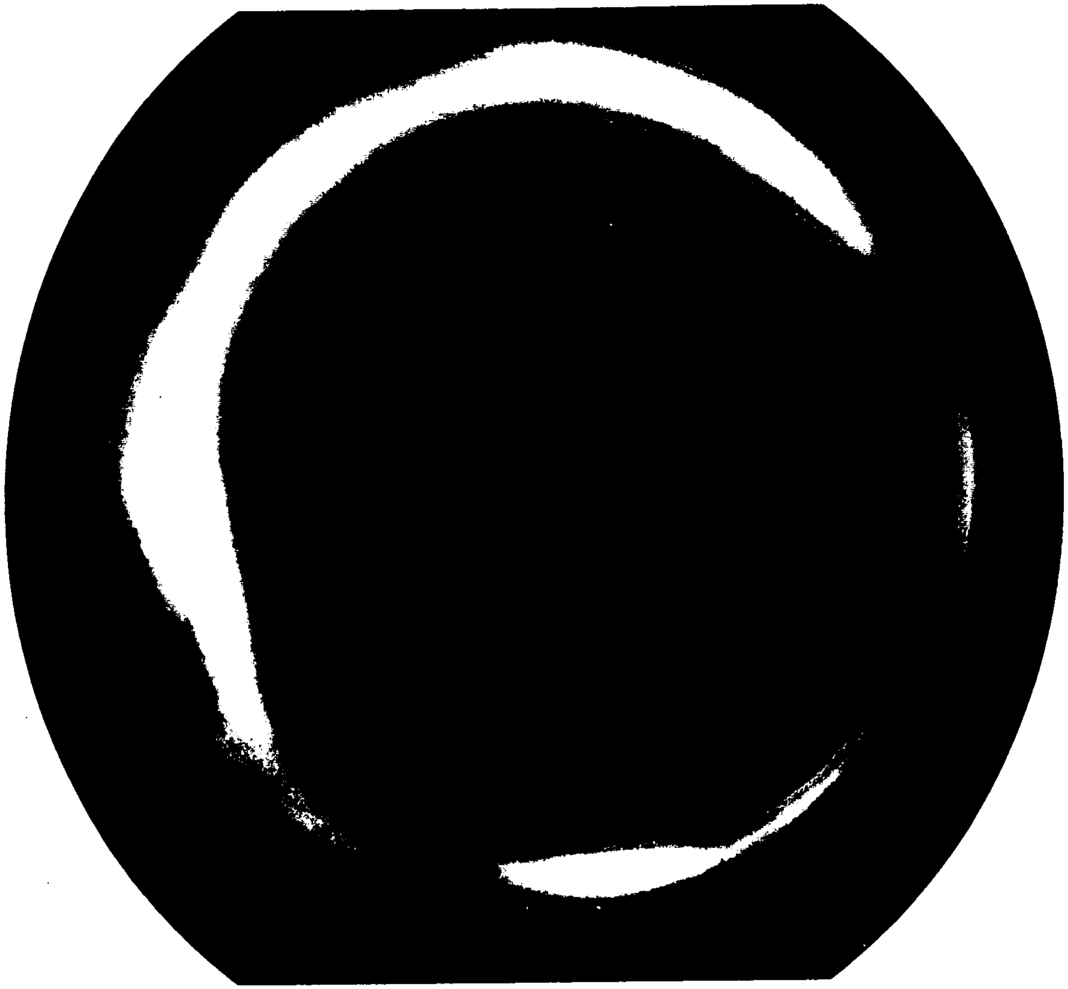


Figure 4

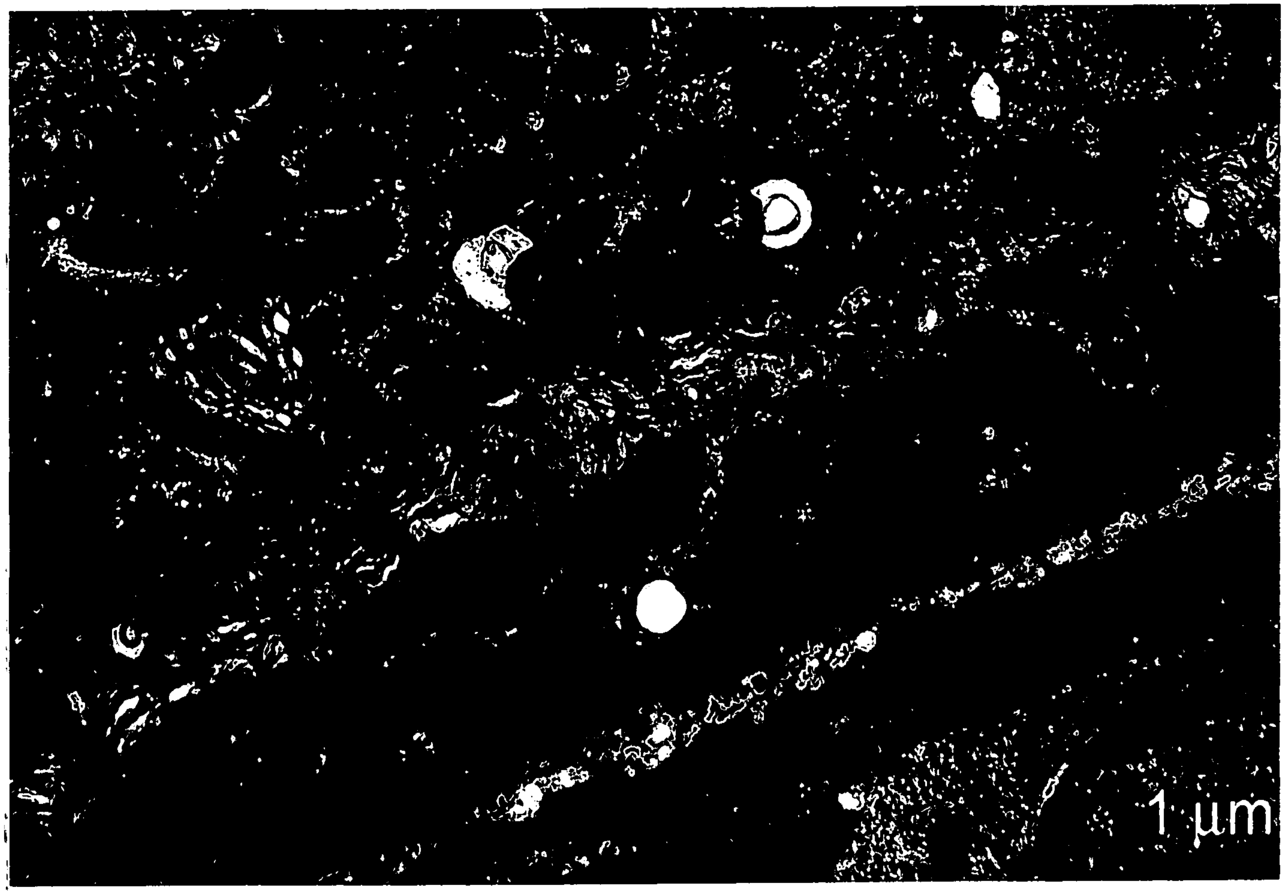


Figure 5

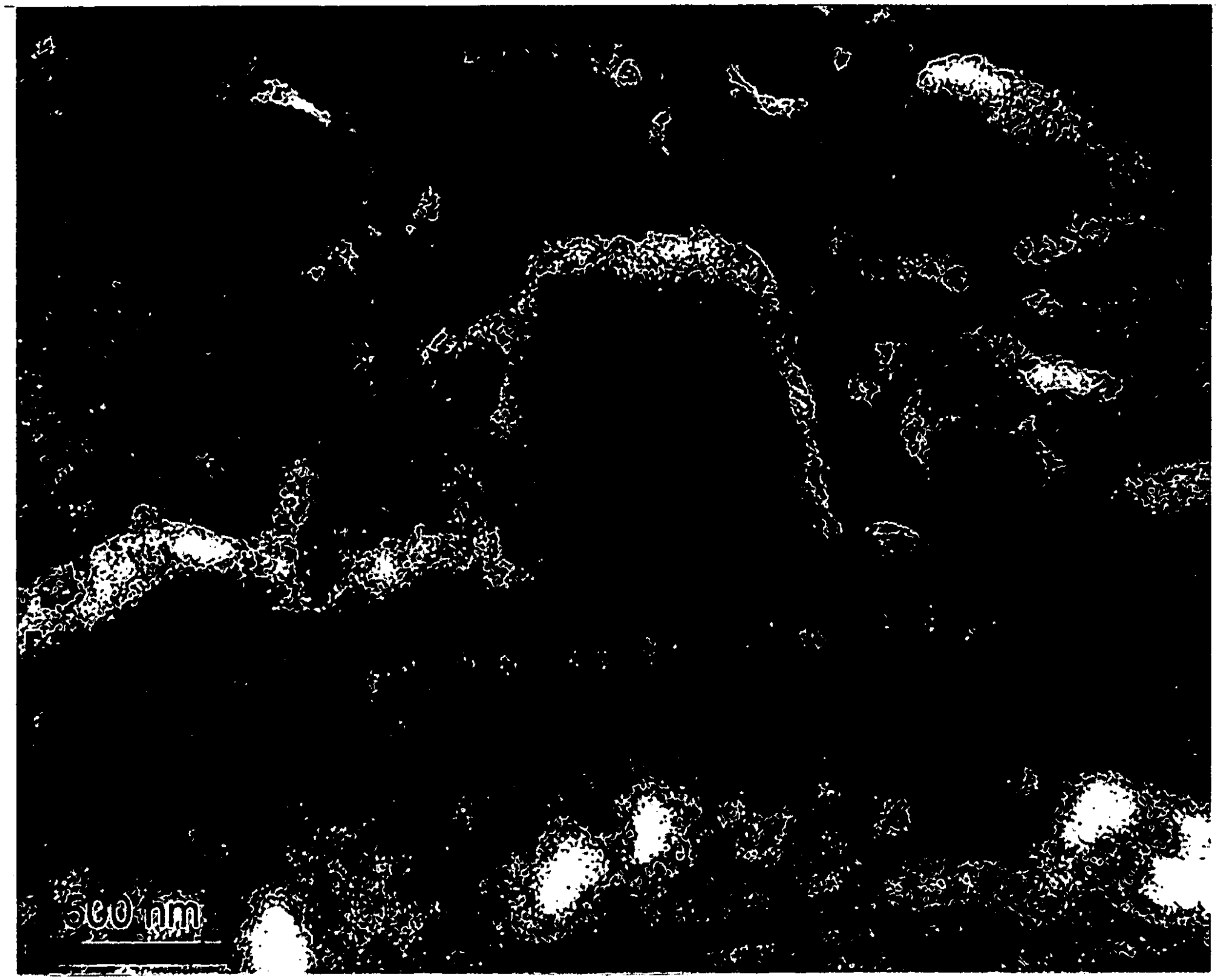


Figure 6